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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference BET 03P0879	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/IB 03/03882	International filing date *(day/month/year)* 12.09.2003	Priority date *(day/month/year)* 13.09.2002
International Patent Classification (IPC) or both national classification and IPC C12N7/00		
Applicant INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE M		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09.02.2004	22.12.2004
Name and mailing address of the International preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Brouns, G Telephone No. +31 70 340-3789

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IB 03/03882

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-60 as originally filed

Claims, Numbers

1-45 as originally filed

Drawings, Sheets

1/6-6/6 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-45
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-45 (no)
Industrial applicability (IA)	Yes:	Claims	1-45
	No:	Claims	

2. Citations and explanations

   see separate sheet

The present application shows the generation of retroviral particles pseudotyped with hepacivirus, more specifically Hepatitis C virus (HCV), E1 and/or E2 genes, whereby the carboxyl terminus of the core protein provides the signal sequence for E1. Said particles can infect both primary hepatocytes and an hepato-carcinoma cell line, provided both E1 and E2 are present, and infection can be neutralised by sera of HCV infected patients. It is suggested to use said particles to identify hepaciviral receptors, in diagnostics to detect neutralising antibodies in patients sera and to develop inhibitors to hepaciviral infection.

1) The following documents (D) are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: MATSUURA YOSHIHARU ET AL: "Characterization of pseudotype VSV possessing HCV envelope proteins" VIROLOGY, RAVEN PRESS, NEW YORK, NY, US, vol. 286, no. 2, 1 August 2001 (2001-08-01), pages 263-275

D2: WO 02 070651 A (HAZUDA DARIA J ;MERCK & CO INC (US); SIMON ADAM J (US); LINEBERGER) 12 September 2002 (2002-09-12)

D3: COCQUEREL LAURENCE ET AL: "Topological changes in the transmembrane domains of hepatitis C virus envelope glycoproteins." THE EMBO JOURNAL. ENGLAND 17 JUN 2002, vol. 21, no. 12, 17 June 2002 (2002-06-17), pages 2893-2902, ISSN: 0261-4189

D4: WO 01 21807 A (EMERSON SUZANNE U ;BUKH JENS (US); US HEALTH (US); FORNS XAVIER (U) 29 March 2001 (2001-03-29)

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Novelty

2) The prior art does not disclose retroviral particles pseudotyped with HCV E1 and E2 proteins, therefore the subject-matter of the present set of claims fulfills the requirements of Article 33(2)PCT.

Inventive step

3.1) Document D1, which is considered to represent the most relevant state of the art

for claim 1, discloses (D1, page 272, column 2; figure 1) a method for producing hepacivirus pseudo-particles *ex vivo*, consisting of Vesicular Stomatitis Virus pseudotyped with HCV E1 and/or E2 proteins comprising signal peptides, as an efficient tool for research on cellular receptors for HCV and for the development of prophylactics and therapeutics for hepatitis C.
From this the subject-matter of claim 1 differs in that a method to produce a retrovirus pseudotyped with hepacivirus E1 and/or E2 proteins is disclosed.

The problem to be solved by the present invention may therefore be regarded as the provision of a method to generate an alternative hepaciviral pseudo-particle as an efficient tool for research on cellular receptors for hepaciviruses and for the development of prophylactics and therapeutics for hepacivirus mediated disease.

The solution proposed in claim 1 of the present application cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

Pseudotyping of retrovirus with a number of different viral glycoproteins using a three plasmid system is well known in the art, and in particular D2 discloses a method of generating a pseudotyped retroviral particle (D2, examples 1 and 2) and suggests to generate a HCV envelope pseudotyped retrovirus (D2, page 12, lines 30-31; claim 18, 32, 41). The skilled person having knowledge of D2 as well as the infectious HCV pseudo-particle of D1 would arrive at a method of producing a hepacivirus-like particle without use of his inventive skill and with reasonable expectation of success.

3.2) Dependent claims 2-13, 20-22, 25-31 and 33-45 do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT with respect inventive step, since these features are well known from the prior art:

The use of the last 21 amino acids of the native hepacivirus core protein, followed by E1, E2 and (part of) p7 is known to be essential for the correct biogenesis of hepacivirus glycoprotein (D3, abstract; figure 1). However, said native E1/E2 glycoprotein complex in D3 remains intracellularly, whereas the E1/E2 complex in the present application appears to be incorporated in retroviral particles budded from the transfected cells. At present, there are no **technical features** disclosed in the claimed method of producing hepacivirus-like particles using a ΔC-E1-E2 construct of the present application that result in the observed surprising technical effect, namely enhanced expression of E1/E2 at the cell surface, where packaging into retroviral

particles is considered to take place.

HIV pseudotyped with HCV, comprising a transgene is known from D2 (D2, claim 18) and the use of HCV pseudotyped HIV particles in screening of molecules interfering with HCV entry is the subject-matter of D2 (D2, claim 41). The different screening methods are routine practise for the skilled person.
D3 (abstract) discloses HCV-like particles expressing E1 and/or E2 that have been used for transferring a transgene to a hepatic cell and teaches that said particles are suitable for development of vaccines, therapeutics, diagnosis of HCV infection and for identification of a HCV receptor.
The deletion mutants of claim 12 and 22 have been described in D4 (examples 1-6).

3.3) No inventive step is acknowledged for the present set of claims (Article 33(3) PCT).

Other remarks

4) It seems to be the object of the present invention to provide an **infectious** hepacivirus particle, whereas the claims also seem to comprise embodiments of viral particles comprising either E1 or E2, which have been shown to have low residual infectivity (figure 2; description page 3, lines 9-11).
Furthermore, D2 discloses that expression of the full length core protein together with E1 and E2 results in intracellular localisation of virus-like particles, which does not solve the problem posed in the present application.
The use of the last 21 amino acids of the core protein, known to be the signal sequence for E1, as well as the presence of both E1 and E2 in the hepaciviral particle seem therefore to be essential technical features to practise all the applications of the present invention.
Since independent claims 1, 14, 25, 29-39 and 43 do not contain these features they do not meet the requirement following from Article 6 and Rule 6.3(a) PCT that any independent claim must contain all the technical features essential to the definition of the invention (PCT Guidelines, 5.33).

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